Molecular chaperones: **How J domains turn on Hsp70s**

William L. Kelley

Molecular chaperones of the heat shock protein 70 (Hsp70) variety facilitate protein folding and assembly. They are assisted in this role by their Hsp40 partners, and recent studies have shed new light on how the 'J domains' of these 'cochaperones' activate substrate binding by Hsp70 molecules.

Address: Département de Biochimie Médicale, CMU-Université de Genève, 1 rue Michel-Servet, 1211 Genève 4, Switzerland.
E-mail: William.Kelley@medecine.unige.ch

Current Biology 1999, 9:R305–R308
http://biomednet.com/elecref/09609822009R0305

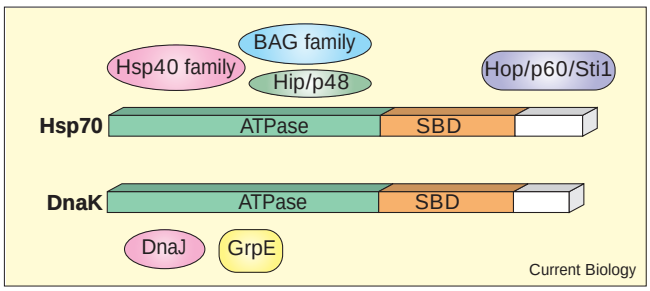
The notion that, inside cells, newly synthesised proteins do not simply fold and assemble into appropriate oligomeric complexes unassisted, but that they are nurtured by 'molecular chaperones', is by now familiar. A number of different classes of molecular chaperones have been defined, and the emerging picture is that these cooperate in the production of mature proteins. One such class is the heat shock protein 70 (Hsp70) family. Hsp70s and their associated cochaperones participate in numerous processes essential to cell survival under both normal and stressed conditions [1]. They assist, for example, protein folding and translocation across membranes, the assembly and disassembly of protein complexes, the presentation of substrates for degradation, and the suppression of protein aggregation. Such versatility is intriguing for a protein machine composed of so few components, and the importance of Hsp70s is underscored by their wide evolutionary conservation.

A key feature of Hsp70 action is that the reversible binding and release of substrate molecules are coupled to a cycle of ATP hydrolysis and conformational change [2,3]. Understanding the details of this ATPase cycle is a prerequisite for understanding how Hsp70 works. In Hsp70's ATP-bound form, its substrates have a low affinity, primarily due to high off rates, whereas in its ADP-bound form, they have a high affinity, primarily due to lower off rates. The intrinsic ATPase activity of Hsp70 proteins is extremely weak, but the steps of the ATPase cycle are regulated by interaction with a number of other types of protein. In particular, members of the Hsp40 family of 'cochaperones' stimulate the Hsp70 ATPase reaction; a cohort of accessory proteins promotes or attenuates the ADP–ATP exchange reaction; and a number of other regulatory factors are emerging, such as the BAG-1 family [4]. Figure 1 summarises Hsp70's domain organisation and known interacting regulatory factors.

Hsp40 cochaperones are defined by an approximately 70 amino-acid 'signature sequence', known as the J domain [5]. There is accumulating evidence that specific members of the Hsp70 family are partnered by particular Hsp40s — that is, only certain members of the Hsp40 family can functionally interact with specific Hsp70 proteins, either as a result of co-localisation in the same subcellular compartment, or by as yet unknown mechanisms that confer specificity on cognate partner recognition [6–9]. A number of recent studies have addressed important questions about this system, such as how and where Hsp40s interact with Hsp70s, and how the interaction leads to the activation of the Hsp70 partner to bind substrates.

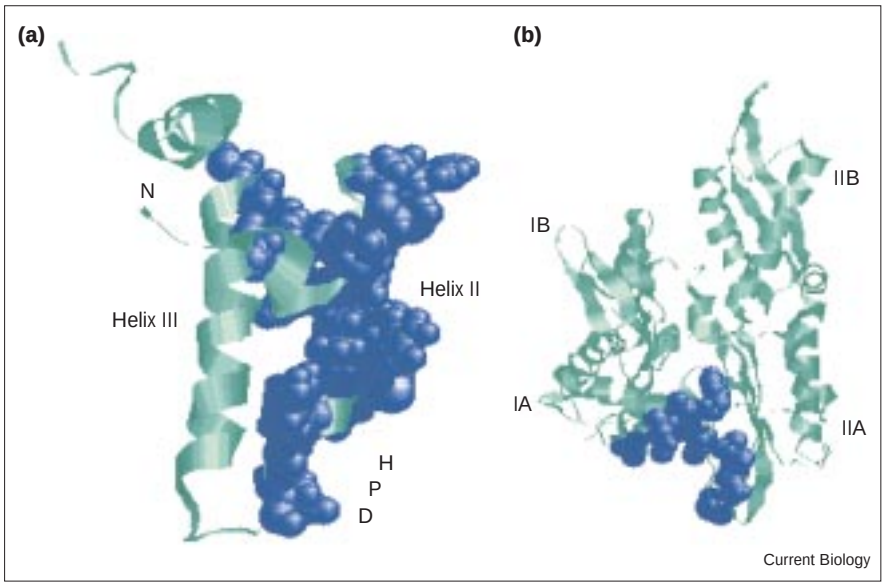
High resolution structures have been obtained for two isolated J domains, two mammalian Hsp70 ATPase domains, the ATPase domain of the *Escherichia coli* Hsp70 DnaK in complex with part of its nucleotide exchange factor GrpE, and a fragment of the DnaK substrate-binding domain. Unfortunately, however, we do not yet have a structure for an intact Hsp40 or Hsp70 molecule, nor for an Hsp40–Hsp70 complex. Other approaches have therefore been taken to identify the sites of Hsp40–Hsp70 interaction. For instance, the J domain is formed from three helices that pack together into a compact structure, with the highly conserved tripeptide histidine–proline–aspartate — HPD in the single-letter amino acid code — exposed in a loop between helices II and III [10]. Mutations of this tripeptide reduce or abolish stimulation of the Hsp70 ATPase reaction, strongly suggesting that the J domain plays an important part in the interaction of an Hsp40 protein with its Hsp70 partner. Direct evidence for this has been difficult to obtain, however.

Figure 1



Typical domains and interacting regulatory factors of (top) a eukaryotic Hsp70 and (bottom) the *Escherichia coli* Hsp70 DnaK. Both types of Hsp70 have a 45 kDa amino-terminal ATPase domain, a 15–18 kDa substrate-binding domain (SBD), and an approximately 10 kDa carboxy-terminal domain of largely unknown function.

Figure 2



(a) A ribbon projection of the J domain of DnaJ, with a space-filling representation of the residues thought to mediate this cochaperone's interaction with the ATPase domain of DnaK [11]. (b) A ribbon projection of the DnaK ATPase domain, with a space-filling representation of residues in the small cleft between subdomains IA and IIA that are thought to form, or influence, an interaction site with DnaJ [14,15]. Note that the projections in (a) and (b) have not been equivalently scaled.

Greene *et al.* [11] have now succeeded in mapping the sites of interaction between DnaK and the J domain of its natural partner, DnaJ, by the technique of nuclear magnetic resonance (NMR) perturbation. They prepared an ^{15}N-labelled version of the J domain from DnaJ, and then monitored the NMR response on titration with either full-length DnaK or just its ATPase domain, complexed with ADP or ATP. The results suggest that the interacting surface on the J domain might be as small as residues 2–35, including helix II and the HPD tripeptide segment (Figure 2a). This result is consistent with structure–function predictions [10], but one cannot exclude the possibility that other parts of DnaJ also interact with DnaK. Indeed, evidence discussed below would suggest that DnaJ might also interact with Hsp70 at a second site either within, or close to, its substrate-binding domain [12–15].

The basis of partner selectivity in J domain interactions is not entirely clear. A key insight came when Schlendstedt *et al.* [6] showed in yeast that swapping the J domain of Sec63 with that from Scj1 did not abolish Sec63-like activity — presumably because Scj1 is normally found in the endoplasmic reticulum (ER) lumen and is adapted to interact with BiP, a resident ER Hsp70. In contrast, swapping Sec63's J domain with that of the cytoplasmic protein Sis1 did abolish Sec63-like activity, though this could be rescued by substituting three residues within the Sis1 J domain with the equivalent amino acids from the Sec63 J domain. The interacting Hsp70 partner is also important, as illustrated by the finding that BiP's function in an *in vitro* protein translocation assay could not be replaced by cytoplasmic or mitochondrial Hsp70s; this probably reflects a failure of interaction with the Sec63 J domain [7,8]. Different Hsp70–Hsp40 pairs can display different protein-folding activities in *in vitro* systems, suggesting that even closely related Hsp40s in the same cellular compartment may have specificity differences [9].

A widely used method of assaying functional Hsp70–Hsp40 interaction is to determine the degree of stimulation of Hsp70 ATPase activity while titrating Hsp40. Some caution is warranted, however, as peptide substrates alone have been reported to stimulate ATPase activity, but usually to a minor extent compared with Hsp40. Such an assay was used to define the minimal sequence of DnaJ necessary to stimulate the DnaK ATPase [12]. The results showed that the J domain alone (residues 1–75) was unable to stimulate DnaK's ATPase activity, but maximal stimulation — similar to that observed with full-length DnaJ — could be attained if the J domain and a peptide substrate were simultaneously present. A lower, but still measurable, level of stimulation was observed, in the absence of peptide substrate, using the J domain together with a short flanking sequence (residues 1–106).

Collectively, these findings indicate that a dual signalling mechanism could operate, in which maximal activation of DnaK involves two simultaneous signals: one arising from interaction with the J domain, and the second from interaction with a flexible peptide that can act as a substrate [12]. The activating signals can evidently be provided by residues on the same cochaperone polypeptide, as in the case of the 106 amino-acid DnaJ fragment or indeed the intact DnaJ molecule. The two-signal hypothesis predicts that there be at least two controlling surfaces on DnaK: one for the J domain interaction, and the other for the second signal interaction, perhaps coincident with the

substrate-binding domain. Where, then, are these interacting surfaces?

The site important for J domain interaction might occur in a small cleft between subdomains IA and IIA of the DnaK ATPase domain (Figure 2b). Substitution by alanines of residues lining both sides of this cleft dramatically altered binding to DnaJ [14]; mutations in the neighborhood of this cleft, or within DnaK's central hydrophobic substrate binding pocket, can also apparently uncouple nucleotide hydrolysis and substrate binding (unpublished data cited in [14]). Interestingly, single-turnover ATPase assays further showed that DnaJ can stimulate full-length DnaK at least 100-fold, but cannot stimulate the isolated ATPase domain.

Binding experiments with immobilised DnaJ, using the surface plasmon resonance method, detected an ATP-dependent association of DnaJ with either full-length or partially carboxy-terminally truncated DnaK, but did not detect any interaction between DnaJ and DnaK's isolated ATPase or substrate-binding domains. Despite the above-mentioned NMR evidence that the J domain of DnaJ interacts with the ATPase domain of DnaK [11], the authors [14] reasoned that, under their experimental conditions and kinetic constraints, the DnaK ATPase domain must be physically associated with the substrate-binding domain for DnaJ to stimulate the ATPase reaction. These results suggest that, either the J domain interaction site spans two domains of DnaK, or interdomain communication within a DnaK molecule is required for efficient and productive DnaJ binding, for instance by altering site conformation or accessibility.

Independently, Suh *et al.* [15] sought to isolate mutations of DnaK that suppress point mutations in the J domain HPD tripeptide. In the case of one J domain mutation, the suppressors mapped in the lower region of the same cleft within the DnaK ATPase domain defined by Gässler *et al.* [14]. Alanine substitution of residues surrounding one such suppressor mutation were found to reduce DnaJ binding. Suh *et al.* [15] also tested a set of point mutations in the substrate-binding domain of DnaK for altered interaction with DnaJ. Notably, two such DnaK mutants, which had previously been shown to have an altered peptide-binding affinity, also displayed reduced DnaJ binding, suggesting that DnaJ might also make contact with DnaK's substrate-binding domain. The results of both studies [14,15] appear to support the dual-signal model by revealing potential regions within both the ATPase and substrate-binding domains of DnaK that affect its interaction with DnaJ.

To test the idea that interactions with J domains activate Hsp70 for binding polypeptide substrates, Misselwitz *et al.* [16] developed an elegant *in vitro* solid-phase system using the Sec63 J domain and the Hsp70 BiP. For the assay, a synthetic hydrophobic peptide was first covalently attached to a chip; purified BiP and nucleotides were then passed over the modified chip, and the kinetics of peptide association and dissociation monitored by surface plasmon resonance. Misselwitz *et al.* [16] found that the BiP–peptide association was much stronger in the presence of ADP than ATP, consistent with the notion that Hsp70s bind to substrates more tightly in their ADP form. They next asked whether the addition of purified Sec63 J domain in solution enhanced BiP binding to peptide: they found that this did not further activate BiP peptide binding even though, in control assays, the J domain strongly stimulated the BiP ATPase reaction, clearly indicating that a productive interaction was taking place. How can one reconcile this finding with the view that Hsp40s activate Hsp70 to bind substrate?

The real technical breakthrough came when the J domain and peptide substrate were co-immobilised on the same chip. The idea was that the J-activated state of BiP might be too short-lived to be detected when the interacting partners are both free to diffuse in solution. Using this approach, Misselwitz *et al.* [16] found that a single J domain can indeed activate multiple BiP molecules to bind peptide substrate in the presence of ATP. Control experiments showed, in the presence of ADP, peptide binding was the same whether or not J domain was present on the chip, suggesting that the stimulation of peptide binding by the J domain was dependent upon nucleotide hydrolysis. Furthermore, activation was not observed when a BiP ATPase domain mutant was used that could bind but not hydrolyse ATP, if the BiP–J domain interaction were impaired by a J domain mutation, or if BiP harbored point mutations in its peptide-binding pocket.

Can BiP be induced by interaction with a J domain to bind a wider range of peptides, stably folded protein substrates even, as might be expected given its known potential for binding large substrates? Interestingly, when other peptides were immobilised on the chip, including stably folded proteins such as lysozyme and cytochrome c, BiP was able to bind to them, provided that it had been activated by ATP and a J domain; the binding was abolished in the absence of the J domain [16]. This assay might be broadly applicable to testing cochaperone regulation of Hsp70 substrate selection.

Although we are still far from having a thorough understanding of the Hsp70 regulatory cycle, the recent results discussed above have provided insights into the regulatory actions of Hsp70–J domain interactions. The interacting surfaces are being identified, some clues to the determinants of the interaction specificity are emerging and hints have been given as to how ATP hydrolysis is coupled to substrate binding. This last point deserves special mention. What is the advantage of coupling two

signals to achieve ATPase stimulation and stable substrate binding? A reasonable hypothesis is that this mechanism optimises substrate selection, ensuring that Hsp70 is perhaps only activated by Hsp40-delivered substrates and thereby avoids futile cycling. Future studies focusing on substrate binding and interdomain coupling by members of Hsp70 family should add considerably to unravelling this fascinating mechanism.

Acknowledgements
This work was supported by a grant from SNSF (grant number FN-31-47283-96 to C. Georgopoulos) and the Canton of Geneva.

References

1. Bukau B, Horwich AL: The Hsp70 and Hsp60 chaperone machines. *Cell* 1998, 92:351-366.
2. Rüdiger S, Buchberger A, Bukau B: Interaction of Hsp70 chaperones with substrates. *Nat Struct Biol* 1997, 4:342-349.
3. Mayer MP, Bukau B: Hsp70 chaperone systems: diversity of cellular functions and mechanism of action. *Biol Chem* 1998, 379:261-268.
4. Takayama S, Xie Z, Reed JC: An evolutionarily conserved family of Hsp70/Hsc70 molecular chaperone regulators. *J Biol Chem* 1999, 274:781-786.
5. Kelley WL: The J-domain family and the recruitment of chaperone power. *Trends Biochem Sci* 1998, 23:222-227.
6. Schlendstedt G, Harris S, Risse B, Lill R, Silver PA: A yeast DnaJ homolog, scj1p, can function in the endoplasmic reticulum with BiP/Kar2p via a conserved domain that specifies interactions with Hsp70s. *J Cell Biol* 1995, 129:979-988.
7. McClellan AJ, Endres JB, Vogel JP, Palazzi D, Rose MD, Brodsky JL: Specific molecular chaperone interactions and an ATP-dependent conformational change are required during posttranslational protein translocation into the yeast ER. *Mol Biol Cell* 1998, 9:3533-3545.
8. Brodsky JL, Bäurele M, Horst M, McClellan AJ: Mitochondrial Hsp70 cannot replace BiP in driving protein translocation into the yeast endoplasmic reticulum. *FEBS Lett* 1998, 435:183-186.
9. Lu Z, Cyr, DM: Protein folding activity of Hsp70 is modified differentially by the Hsp40 co-chaperones Sis1 and Ydj1. *J Biol Chem* 1998, 273:27824-27830.
10. Pellecchia M, Szyperski T, Wall D, Georgopoulos C, Wüthrich K: NMR structure of the J-domain and the Gly/Phe-rich region of the ***Escherichia coli*** DnaJ chaperone. *J Mol Biol* 1996, 260:236-250.
11. Greene MK, Maskos K, Landry SJ: Role of the J-domain in the cooperation of Hsp40 with Hsp70. *Proc Natl Acad Sci USA* 1998, 95:6108-6113.
12. Karzai AW, McMacken R: A bipartite signalling mechanism involved in DnaJ-mediated activation of the ***Escherichia coli*** DnaK protein. *J Biol Chem* 1996, 271:11236-11246.
13. Demand J, Lüders J, Höhfeld J: The carboxy-terminal domain of Hsc70 provides binding sites for a distinct set of chaperone cofactors. *Mol Cell Biol* 1998, 18:2023-2028.
14. Gässler CS, Buchberger A, Laufen T, Mayer MP, Schröder H, Valencia A, Bukau B: Mutations in the DnaK chaperone affecting interaction with the DnaJ cochaperone. *Proc Natl Acad Sci USA* 1998, 95:15229-15234.
15. Suh W-C, Burkholder WF, Lu CZ, Gottesman ME, Gross CA: Interaction of the Hsp70 molecular chaperone, DnaK, with its cochaperone DnaJ. *Proc Natl Acad Sci USA* 1998, 95:15223-15228.
16. Misselwitz B, Staeck O, Rapoport TA: J proteins catalytically activate Hsp70 molecules to trap a wide range of peptide sequences. *Mol Cell* 1998, 2:593-603.